Research Exam Q7, biomedical sciences – 2017-2018

April 11, 2018

During the exam you have access on a computer to these books:

Casarett & Doull's Essentials of Toxicology (3e);

You are allowed to use a calculator of the type Casio FX-82MS.

The questions must be answered in English. If you cannot remember a specific English term, you may use the Dutch term.

Name: **Studentnummer:** | | | | | | | |

General question

Question 1 (10 pts)

The Health Council provides advice to the government regarding public health matters. This involves complex decision making based on factual and normative (un)certainties. The text below, which is taken from the work programme of 2018, describes a request for advice from the Minister of Health and Welfare and Sports.

Organ donation following euthanasia: protocol for determining death

In recent years there has been an increase in the number of organ donations following euthanasia in the Netherlands. This development requires a responsible combination of two procedures, namely the procedure for euthanasia and the procedure for postmortal organ donation. At the request of the Minister of Health and Welfare and Sport a working group of the Dutch Transplant Foundation has drawn up an initial version of a guideline on how that combination procedure can be implemented with maximum care and safeguards. In this context there is a need for a specific protocol to determine death. The Health Council did not consider this issue in the advisory report entitled 'Determining death in postmortal organ donation' (2015/13(R)). The Minister has now submitted an explicit request for advice to the Council so that a legal basis can be created.

The table below allows the classification of such a request based on certainty pertaining normative criteria and the knowledge about the underlying health issue.

	HIGH certainty on knowledge	LOW certainty on knowledge
HIGH consensus on normative criteria	A	B
LOW consensus on normative criteria	C	D

Which cell (A, B, C or D) of this table provides the best classification of the issue of determining death in the context of organ donation after euthanasia? Explain your answer with one argument regarding the certainty of knowledge and one argument regarding the consensus on normative criteria surrounding this issue.

Correct cell (4 pt): B.

Argument on normative criteria (3 pt): There is a guideline on the subject of organ donation following euthanasia and this suggests that there is a high consensus on normative criteria and no crucial moral arguments to reject postmortal organ donation after euthanasia.

Argument on knowledge (3 pt): New uncertainties have arisen with regard to knowledge: how can we determine death when organ donation takes place after euthanasia?

Vaccination

Question 2 (10 pts)

The MMR (Measles Mumps Rubella) vaccine is a live attenuated vaccine containing three viruses. Children are vaccinated at the age of 14 months and get a second vaccination when they are 9 years old.

a. State a major advantage and a major disadvantage of the use of live attenuated vaccines. Explain why this is a major advantage or a disadvantage and explain this. (4 pts)

b. Describe essential steps in the induction of an immune response by a vaccine after injection.. Use at least the following words in your answer: Antigen presenting cell, T cell, B cell (4 pts)

c. Explain the main difference in the induction of an immune response by a killed viral vaccine and a live attenuated viral vaccine. Explain how this influences the protection of the vaccine.(2 pts)

a)

advantage: it is live, so it infects cells and therefore induces a stronger/broader immune reponse

disadvantage: cannot be used in immunocompromised patients/ it is a live attenuated vaccine, what if its reverts to its virulent form again

b)
Infection APC + recognized by PRR, inflammation and maturation APC. APC travels to lymph node, activates T-cells (+ B-cells), induction effector immune response, induction memory cells.

c)
Live vaccine infects cells, killed not. Different activation TLR's: different induction immune response (more cellular induction), antigens are longer present: stronger induction immune response

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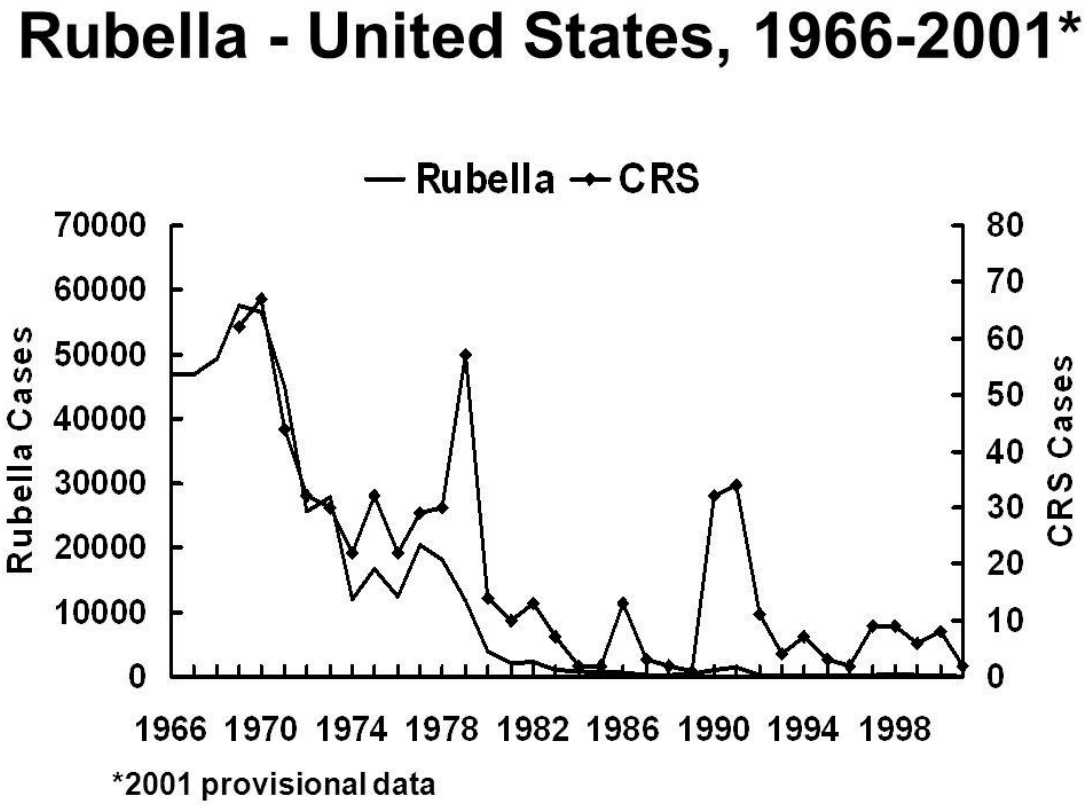
Question 3 (10 pts)

Rubella is a mild childhood disease. Humans are the only host. The virus is transmitted by the respiratory route and replicates in the nasal tissue. The biggest problem is that infection during early pregnancy may cause death of the fetus or Congenital Rubella Syndrome, with severe birth defects in eyes, heart and brain. The critical vaccine coverage for Rubella is 84%.



a. In the figure above you see the incidence in Rubella and Congenital Rubella syndrome (CRS) in the United States. After Rubella vaccination started in 1969 in the United States, a significant decrease in the incidence of Rubella was seen. Explain why an increase in congenital rubella syndrome can be seen if the critical vaccine coverage is not reached. (4 pts)

b. The Rubella vaccine has been given to girls in the Netherlands since 1974. Explain why it was decided that also young boys should be vaccinated, which was implemented in 1987. (3 pts)

c. The vaccine coverage in the Netherlands is about 94%. Explain why we incidentally still see a small outbreak of Rubella in the Netherlands. (3 pts)

a)
Small outbreaks will occur and can infect not vaccinated pregnant women who have not been in contact with the disease (less change to be infected before child bearing age because of vaccination, so more change of developing congenital rubella syndrome)

b)
Herd immunity

c)
Coverage is not uniform in the Netherlands. In communities with lower coverage (e.g. bible belt) we sometimes see infections.

Question 4 (10 pts)

A correlate of protection is not established thus far for may vaccines and diseases, but knowing these would have major advantages.

a. Explain what is meant by a correlate of protection. (3 pts)

b. Name an advantage of the use of a correlate of protection when testing a vaccine and explain how this can be used. (3 pts)

c. Describe how a correlate of protection can be established for a vaccine in human use. (4 pts)

a)

Biomarker statistically associated with protection against disease

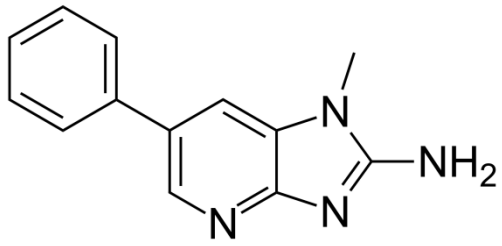
b)
Predict efficacy. Substitute endpoint without exposure/infection.

c)
Vaccination RCT, always including disease (natural or induced), measure immunological markers and correlate these with protection against disease

Risk assessment

Question 5 (15 points)

PhIP (2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) is abundant in cooked meat under typical grilling conditions. The international Agency for Research on Cancer (IARC) has classified PhIP as possibly carcinogenic to humans.



a. Explain for each of the four methods to assess the toxicity of chemicals how they can be used to identify the hazard of PhiP. (3 pts)

b. PhiP is oxidized by CYP1A2 into a carcinogenic N-oxidation product at the amino group. Draw this product. (4 pts)

c. PhiP can be conjugated by Phase II metabolite in an N-glucuronide. Is this a toxification or detoxication reaction? Explain your answer. (2 pts)

d. In a toxicokinetic study PhiP was administered by gavage (oral route) to mice in a dose of 40 ng/kg. Over the course of the experiment the plasmaconcentration-time curve of PhiP revealed an AUC of 5 ng*min/mL. After administration of 20 ng/kg PhiP intravenously an AUC of 100 ng*min/mL was found. Calculate the bioavailability of PhiP in these mice (3 pts).

e. Which group of people is at risk for PhIP toxicity and what factors determine if they will develop cancer due to PhIP? (3 pts)

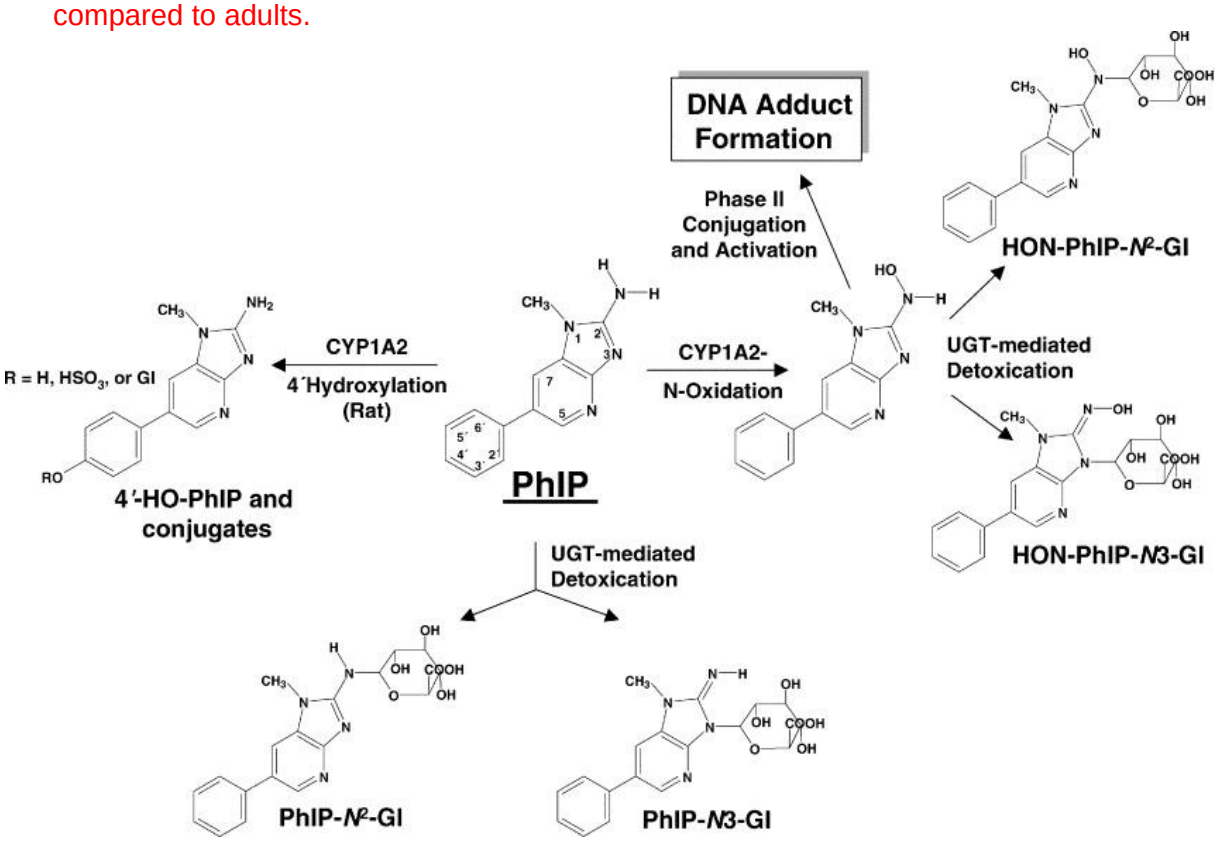
Answers

a. SAR - predict the toxic intermediate.
In vitro assays – only when correct biotransformation enzymes are present

Animal bioassays – if power is sufficient (high dose) this would be detected.

Epi – very difficult to compare groups that are exposed to high and low concentrations due to high background.

b. See N-oxidation step in figure below.
c. Glucuronidation prevents DNA adduct formation of the reactive PhiP N-oxide metabolite.
d. F = AUCpo/AUCiv*(Div/Dpo) = 5/100 * (20/40) = 0.025 = 2.5%
e. Groups that eat meat (especially BBQ and grilled meat). Depends on how certain carcinogenic PhiP actually is in humans ("possible carcinogen") and what the actual exposure (dose-response) is. Children might be more at risk because of a relatively higher exposure as compared to adults.



Question 6 (15 points)

Formaldehyde in anatomy pathology

Formaldehyde is a small molecule (CH_2O) with strong irritating and sensitizing properties. It is used in 3-4 % dilutions in water (formalin), as fixative and preservative in anatomy and pathology departments of hospitals and universities. Exposure to formaldehyde can lead to respiratory and skin sensitization. A suggested mechanism for the observed cytogenetic damage is related to genotoxicity, including the formation of protein and DNA crosslinks. There is sufficient evidence for carcinogenicity of nasal tissues but for tumors of internal organs, more specifically of the hematological malignancies (leukemia's), the evidence is rated as 'limited' by the WHO. Overall, formaldehyde is classified as a carcinogen to humans. The available human data are not sufficient to make a reliable judgement on the reproductive toxicity of formaldehyde exposures.

a. Which three general approaches can be applied to characterize the exposure to formaldehyde? (3 pts)

b. What are the two most important sources of uncertainty in a quantitative risk assessment for reproduction toxic endpoints? (3 pts)

c. Which factors will determine the risk during anatomy classes? Provide three environmental factors and three host factors. (6pts)

d. Based on the information provided, how would you describe the *hazard* of formaldehyde in a communication to the students who follow anatomy classes involving formaline-immersed preparations? Use 2-3 short sentences. (3 points)

Answers

a. Questionnaires, measurements and modeling
b. For this endpoint human are not sufficient. The risk assessment should then be based on animal data. The most important sources of uncertainty are extrapolation from animal to humans (inter species) and differences in susceptibility within the population (intra-species).
c. Environmental factors: size of preparation/amount of formaline used, air temperature, volume and air exchange rate in room.
   Host factors: physical activity level, gender, BMI, genotype and phenotype of enzymes involved in detoxifying metabolism.
d. Formaldehyde is an irritant and sensitizing substance. It is a confirmed human carcinogen. It is not known if and to what extent formaldehyde is reprotoxic in humans.

Screening

Question 7 (4 pts)

Newborn screening is conducted in the first week of life on a few drops of blood from the heel or pulse of a baby. At this moment, newborn screening in the Netherlands focuses on 19 treatable diseases, which are almost all genetic diseases. The purpose of newborn screening in the Netherlands is to detect diseases early in the life of an infant, in order to allow an early start of treatment which contributes to a better prognosis.

Newborn screening is usually conducted with biochemical screening technologies such as tandem mass spectrometry and high-performance liquid chromatography. However, it is expected that it will be more efficient and cheaper to conduct screening with innovative next generation sequencing technologies (NGST) in the near future. This expected shift in the technology used for screening raises ethical questions. Give two examples of ethical questions raised by this shift towards NGST. Explain why they are connected to the use of NGST.

Possible answers:

- NGS raises the question whether and how the newborn screening program should be extended. And if it is extended, on what diseases or disease-information should it focus? Should NBS continue to target treatable diseases? Or should it also return other types of information to parents, such as information about untreatable diseases, risk information, information about late-onset diseases or information about carrier status? (Justifying extending the program)
- NGS can offer a lot of disease-related information about newborn children. While it is possible to filter the information that NGS produces in order to make it focus only on the diseases included in the NBS program, there is always a risk that it will produce secondary findings as well. Secondary findings are findings that are unsought for, but which NBS with NGS produces nevertheless. How should secondary findings be dealt with? Should they be returned to parents or not? And on the basis of what arguments can professionals in NBS justify it for themselves to not return these findings to parents? (the possibility of doing harm through secondary findings)
- NBS is a voluntary program in the Netherlands. Parents receive information about NBS in the last phase of pregnancy and decide on the basis of that information whether they want to have their child screened or not. It is questionable, however, whether NBS can continue to be voluntary if the program extends as an effect of the use of NGS and includes more diseases that are not treatable, or may even only be diagnosed in adulthood. If the NBS program enlarges, the information that must be communicated to parents prior to NBS becomes more complex and voluminous. Can parents understand and digest that information and base a decision on it? And if the answer to that question is ‘no’, is it then possible at all to continue to make participation in NBS voluntary? (The problem of informed consent, which is related to autonomy)
- Is it possible to continue to make NBS comply with the criteria by Wilson and Jungner? If NBS is no longer voluntary, if screening detects not only treatable diseases but also other disease-information and if it produces secondary findings, will it still comply with the Wilson and Jungner criteria? Or do these criteria need a revision? (here, the issue of voluntariness – respect for autonomy – is at stake, furthermore this seems to promote medicalization. And, do benefits outweigh harm here?)

Question 8 (16 points)

One of the diseases that is NOT included in the newborn screening in the Netherlands is Duchenne muscular dystrophy (DMD), a progressive X-linked neuromuscular disorder. It has an estimated worldwide incidence of 1:3500 male births. Currently, there are no curative treatments and the mean age of diagnosis is 5 years. Mothers often are pregnant again before the diagnosis of DMD in the newborn. In Wales, United Kingdom, a screening programme was introduced in 1990. Newborn bloodspots were collected routinely as part of the Wales newborn screening programme. During a 21-year period, 343,170 children were screened using a bloodspot creatine kinase (CK) assay. A total of 145 cases had a raised CK activity (Z250 U/l). The final diagnosis after a positive screening test was made after 6–8 weeks of follow-up. 79 cases had a normal serum CK, indicating absence of DMD, while 66 cases had an elevated serum CK, leading to a diagnosis of DMD by genotyping or muscle biopsy studies. This long-term study has so far identified 13 cases of DMD that had a negative CK assay.

a. What is the programme sensitivity, specificity, predictive value of a positive test (PV+), and the detection rate? Please write also down how you calculated the measures.(10 pts)

b. Discuss one advantage and one disadvantage of including DMD screening in the newborn screening. (4 pts)

c. The incidence of DMD in Wales was 1:5136 during the period that DMD testing was included in the NBS screening programme. Before DMD screening was included in the newborn screening programme in Wales it was 1:4046. Give an explanation for this drop in incidence. (2 pts)

a) *Programme sensitivity = 66/(66+13 FN results)*100% = 66/79*100% = 83.5%*
This makes the false negative rate 100-83.5 = 16.5%
*Specificity = 343012/343091 * 100% = 99.98%*
PV+ = 66/145 = 45.5%.
Detection rate is 66/343170 = 0.19 ‰

Wales screening programme	Duchenne Muscular dystrophy		
	yes	no	Total
CK assay+	66	79	145
CK assay-	13	343012	343025
total	79	343091	343170

b)

Advantages:
- *Possible reduction of the diagnostic delay*
- *Allows for planning of care of the affected boy*
- *Test performs quite well*
- *Permits parents to reproductive choice*

Disadvantages
- *No curative treatment available for the affected boy*
- *Longer knowledge of an untreatable disease, overshadowing the first months to years of the newborns life (loss of 'golden years')*
- *Violates the newborn's right on an open future*

c)
- *A real drop in the underlying incidence*
- *Parents of affected boys that have been diagnosed after screening may have carried out prenatal testing and subsequent abortion of diseased fetuses.*